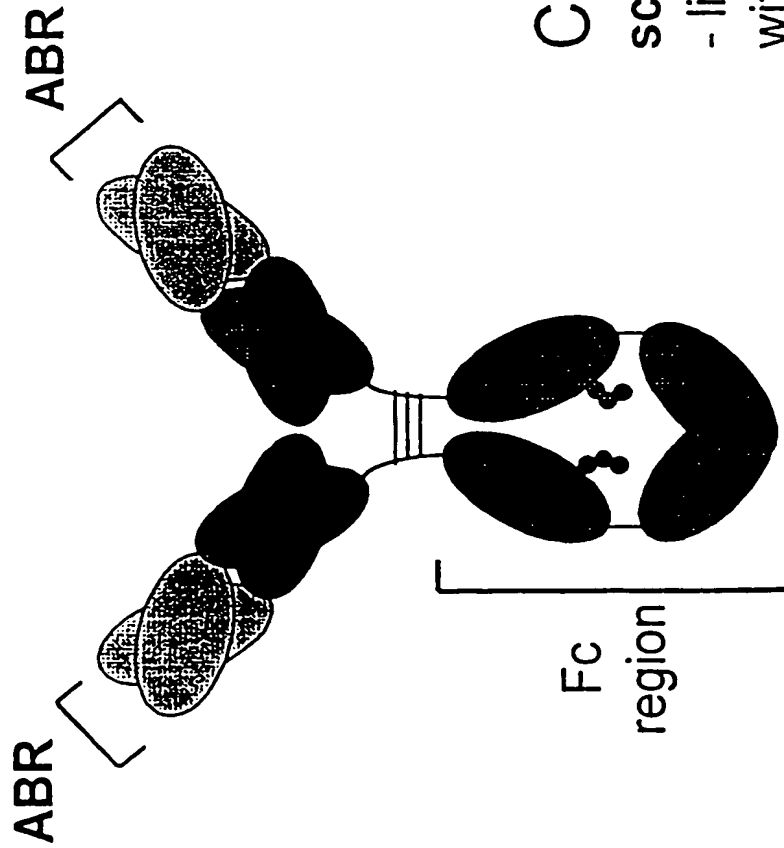


IgG and binding Fragments

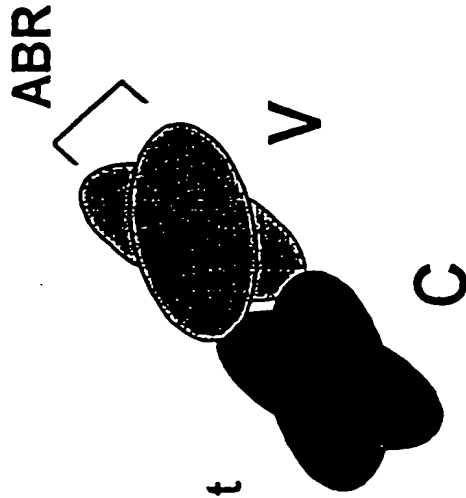
A) Intact IgG



B)

Fab fragment

- $V_L C_L$
- $V_H C_H$
- S=S linked



C)

scFv fragment

- linked V regions with synthetic linker $(Gly_4Ser)_3$

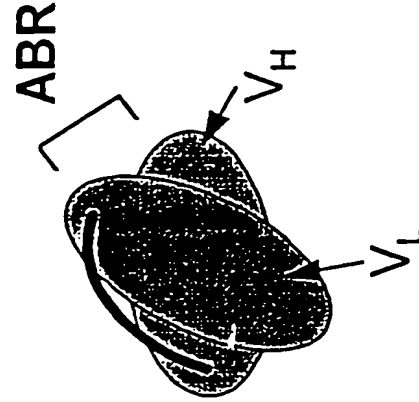


Figure 1

Recombinant Positive Control Reagent

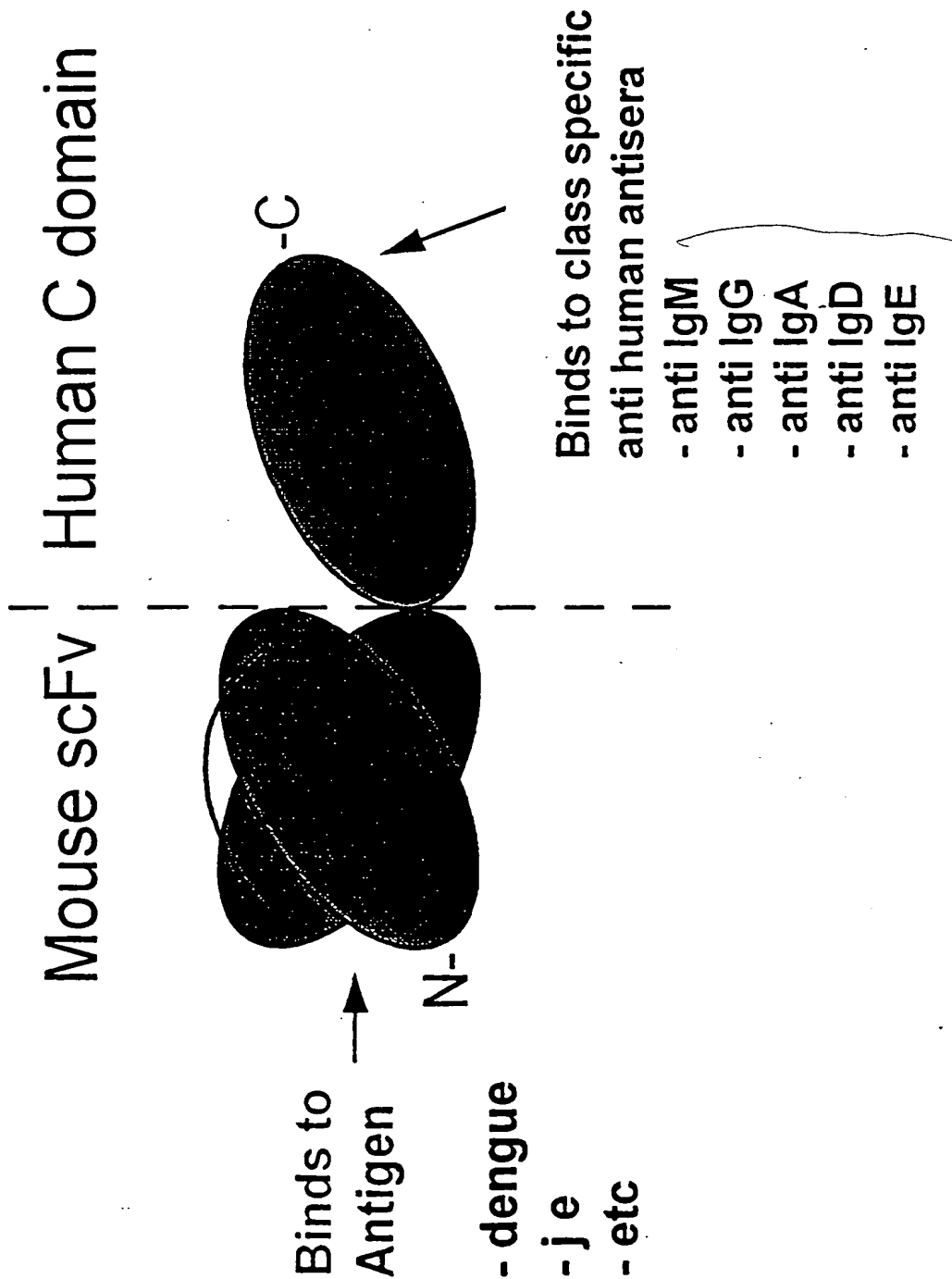


Figure 2

Figure 3

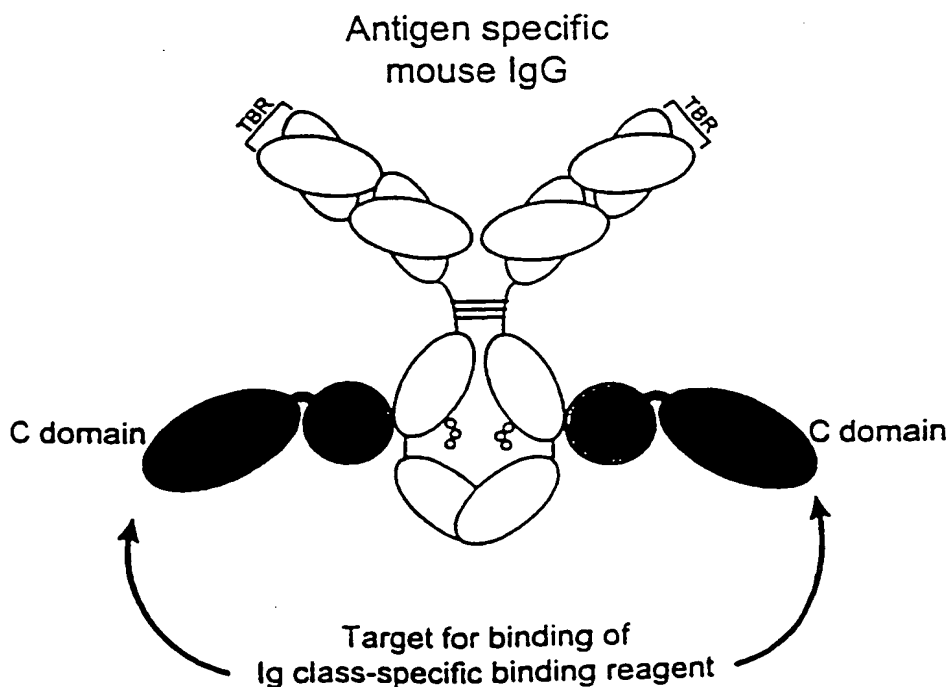
Region 1

Region 2

binds to
Mouse IgG

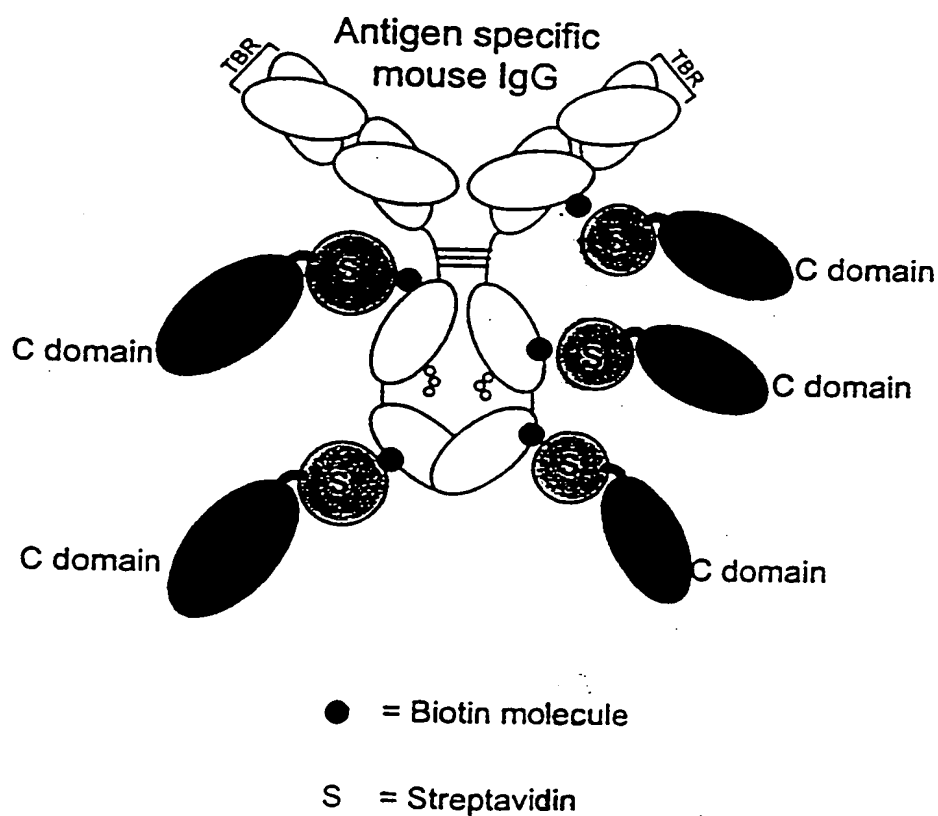
Immunoglobulin C domain
reactive to class specific
anti immunoglobulin binding reagent

Complex formed between bifunctional
molecule and mouse IgG



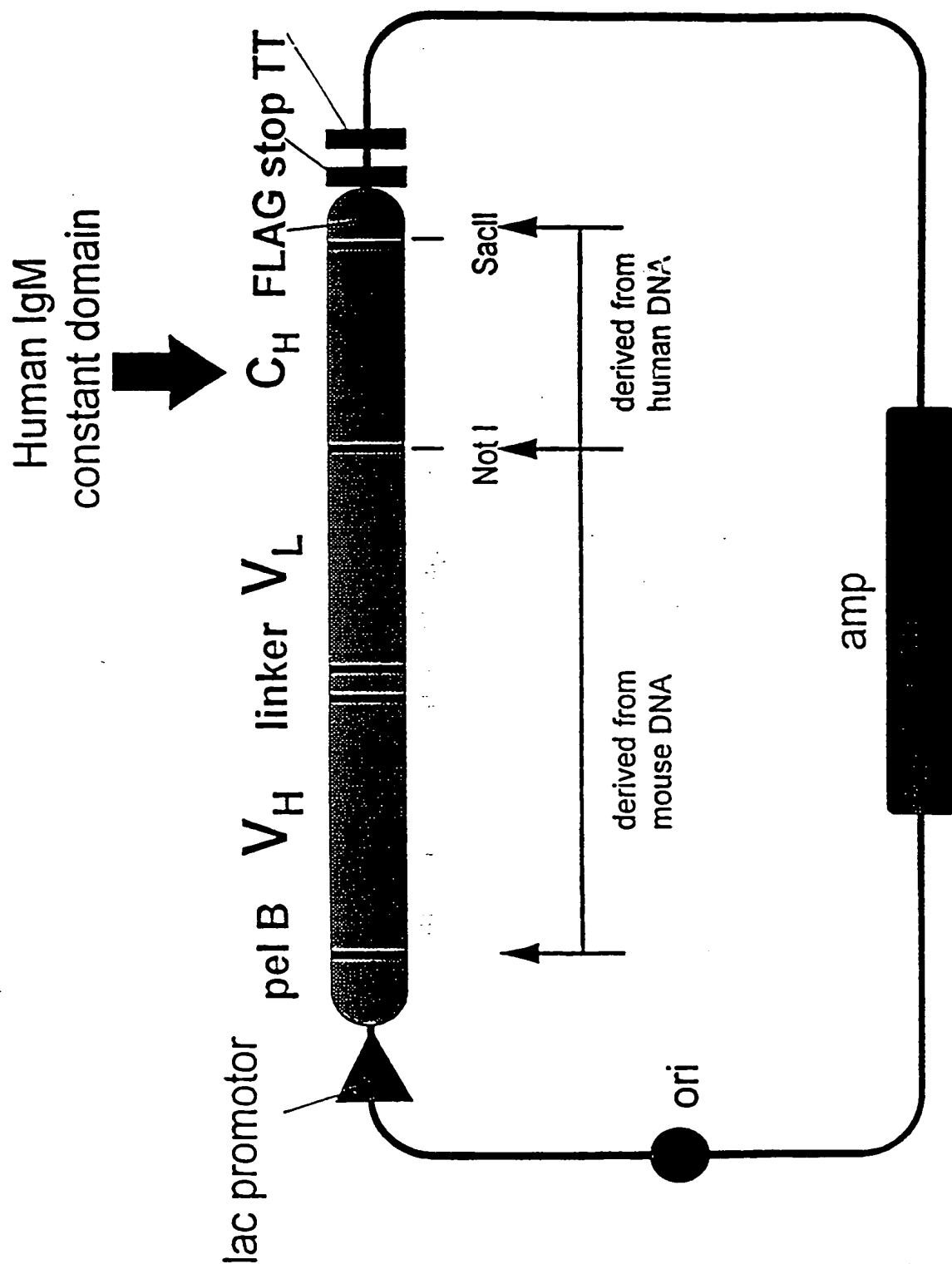
09581924, 061900

**Complex formed between bifunctional
molecule containing streptavidin
and biotinylated mouse IgG**



005790"426T8560

Figure 5



006T90"426T8560

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Figure 6

ELISA reactivity of 1C3- μ domain chimeras

Glycophorin on Plate: Probed with sheep anti Human IgM HRP

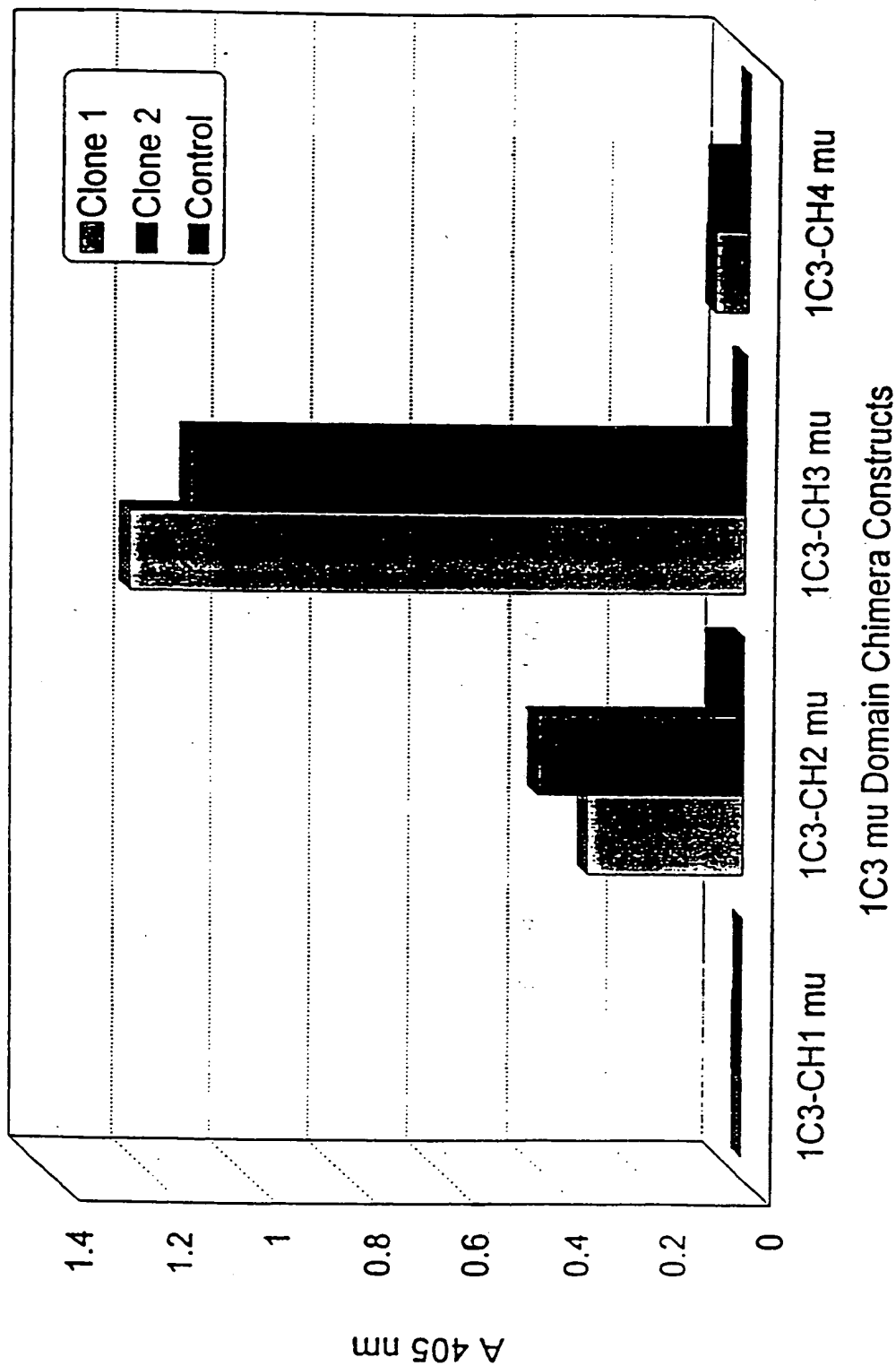


Figure 7

Dengue IgM Capture ELISA

Reactivity of 13C11-CH3 μ protein from *E coli* TOPP6 periplasm

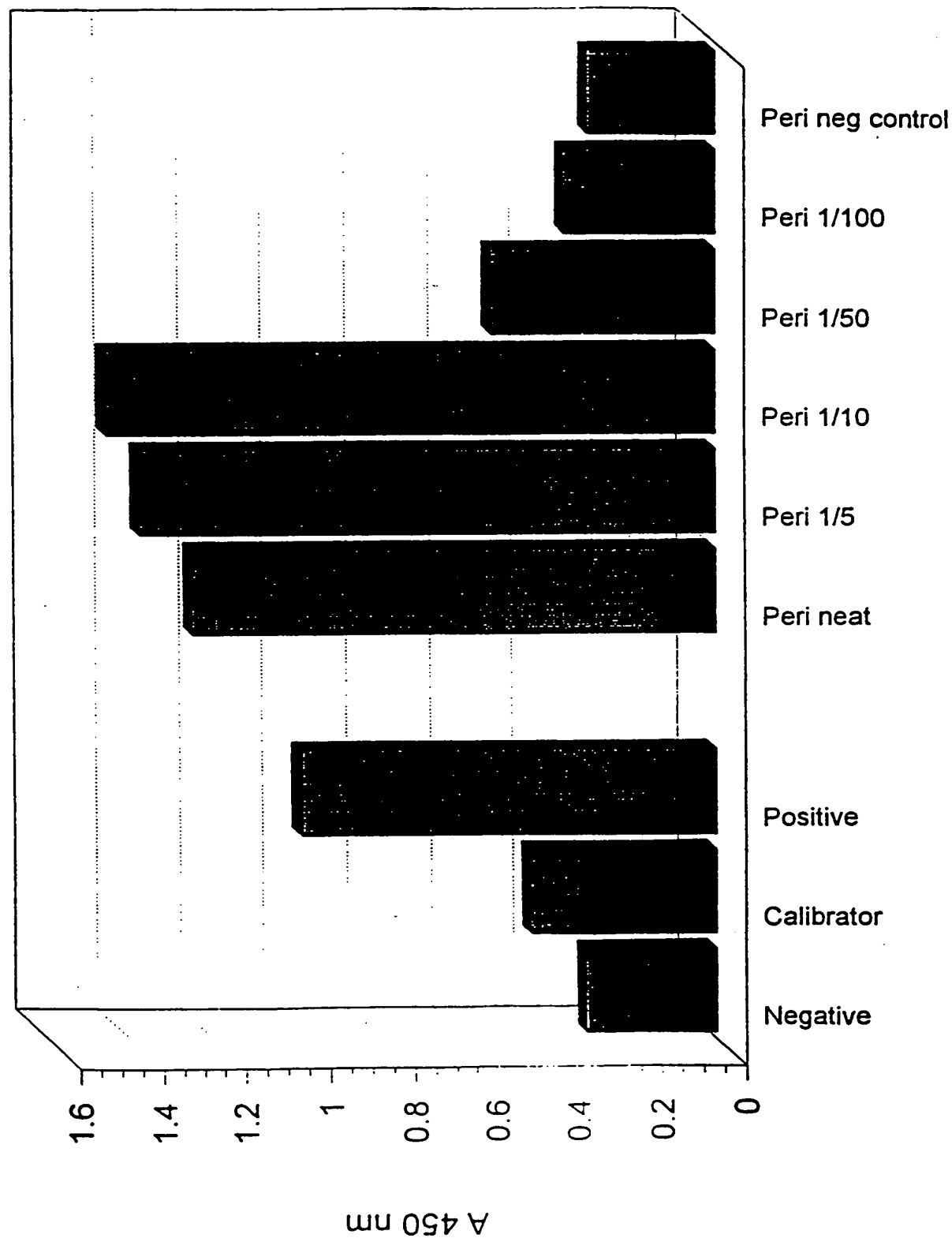
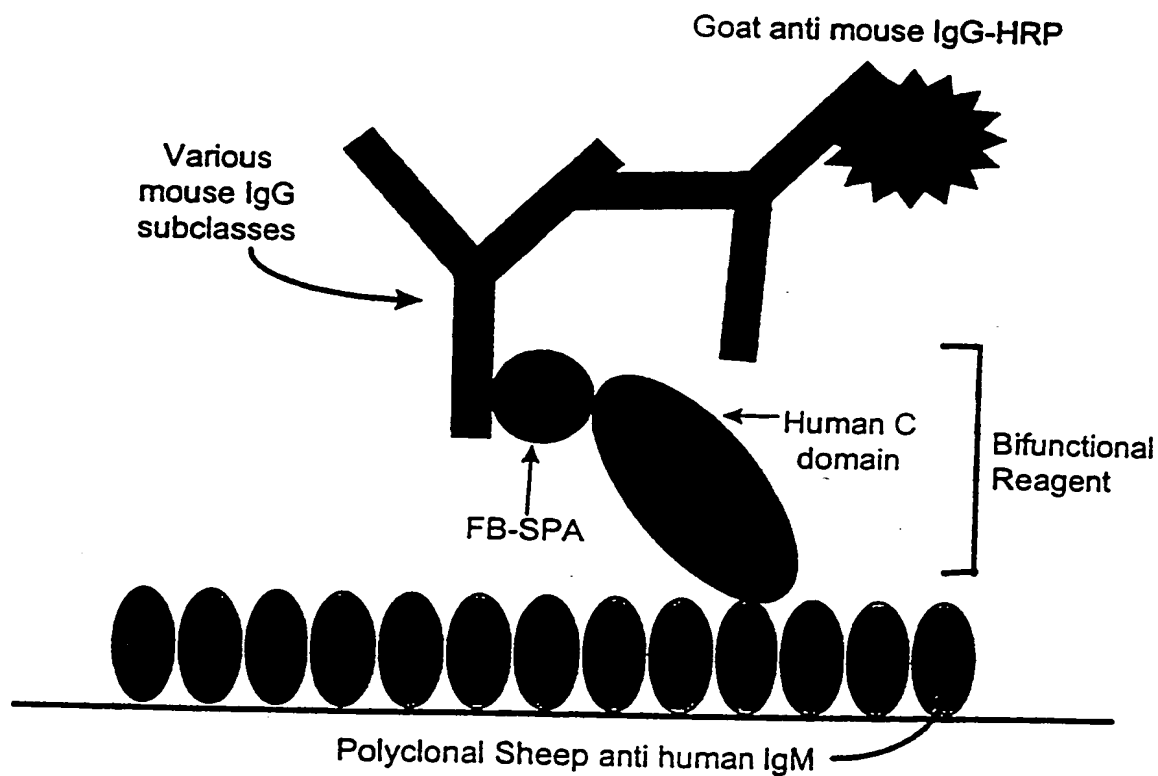


Figure 8

2353 M K Y L L P T A A A G L L L L A
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG
 \--- Pel B-- --->
 2401 A Q P A M A A D N K F N K E Q Q
 GCC CAG CCG GCC ATG GCC GCG GAT AAC AAA TTC AAC AAA GAA CAA CAA
 --- Sfi 1 <N col > < Start Fragment B
 2449 N A F Y E I L H L P N L N E E Q
 AAT GCT TTC TAT GAA ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA
 2497 R N G F I Q S L K D D P S Q S A
 CGC AAT GGT TTC ATC CAA AGC CTA AAA GAT GAC CCA AGC CAA AGC GCT
 2545 N L L A E A K K L N D A Q A P K
 AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GAT GCT CAA GCA CCA AAA
 End Fragment B->
 2593 S D P A A A D Q D T A I R V F A
 AGT GAT CCC GCG GCC GCA GAT CAA GAC ACA GCC ATC CGG GTC TTC GCC
 < linker > Not1 > CH3 mu domain
 2641 I P P S F A S I F L T K S T K L
 ATC CCC CCA TCC TTT GCC AGC ATC TTC CTC ACC AAG TCC ACC AAG TTG
 2689 T C L V T D L T T Y D S V T I S
 ACC TGC CTG GTC ACA GAC CTG ACC ACC TAT GAC AGC GTG ACC ATC TCC
 2737 W T R Q N G E A V K T H T N I S
 TGG ACC CGC CAG AAT GGC GAA GCT GTG AAA ACC CAC ACC AAC ATC TCC
 2785 E S H P N A T F S A V G E A S I
 GAG AGC CAC CCC AAT GCC ACT TTC AGC GCC GTG GGT GAG GCC AGC ATC
 2833 C E D D W N S G E R F T C T V T
 TGC GAG GAT GAC TGG AAC TCC GGG GAG AGG TTC ACG TGC ACC GTG ACC
 2881 H T D L P S P L K Q T I S R P K
 CAC ACA GAC CTG CCC TCG CCA CTG AAG CAG ACC ATC TCC CGG CCC AAG
 2929 G A A D Y K D D D D K *
 GGc GCC GCG GAT TAT AAA GAT GAT GAT GAT AAA TAA GAA TTC AGC CCG
 Sac 2 ----- FLAG ----- Eco R1 <-----
 2977 CCT AAT GAG CGG GCT TTT TTT TAA TTC ACT GGC CGT CGT TTT ACA ACG
 ----- TrpA terminator ----->

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Figure 9



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Figure 10

Sequence of expression cassette Str-C_H3μ in pGC vector

5 M K Y L L P T A A A G L L L L A
 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG
 |--- Pel B-- -->
 A Q P A M A E A G I T G T W Y N
 GCC CAG CCG GCC ATG GCC gag gcc ggc atc acc ggc acc tgg tac aac
 10 --- Sfi I <Nco I >-----core streptavidin ----->
 Q L G S T F I V T A G A D G A L
 cag ctc ggc tcg acc ttc atc gtg acc gcg ggc gcc gac ggc gcc ctg
 T G T Y E S A V G N A E S R Y V
 15 acc gga acc tac gag tcg gcc gtc ggc aac gcc gag agc cgc tac gtc
 L T G R Y D S A P A T D G S G T
 ctg acc ggt cgt tac gac agc gcc ccg gcc acc gac ggc agc ggc acc
 20 A L G W T V A W K N N Y R N A H
 gcc ctc ggt tgg acg gtg gcc tgg aag aat aac tac cgc aac gcc cac
 S A T T W S G Q Y V G G A E A R
 25 tcc gcg acc acg tgg agc ggc cag tac gtc ggc ggc gcc gag gcg agg
 I N T Q W L L T S G T T E A N A
 atc aac acc cag tgg ctg ctg acc tcc ggc acc acc gag gcc aac gcc
 W K S T L V G H D T F T K V K P
 30 tgg aag tcc acg ctg gtc ggc cac gac acc ttc acc aag gtg aag ccg
 S A A S D P A A A D Q D T A I R
 tcc gcc gct agc gat ccc gcg gcc gca gat caa gac aca gcc atc cgg
 strep-| -----< linker > <-Not I > |-----C_H3μ domain
 35 V F A I P P S F A S I F L T K S
 gtc ttc gcc atc ccc cca tcc ttt gcc agc atc ttc ctc acc aag tcc
 T K L T C L V T D L T T Y D S V
 40 acc aag ttg acc tgc ctg gtc aca gac ctg acc acc tat gac agc gtg
 T I S W T R Q N G E A V K T H T
 acc atc tcc tgg acc cgc cag aat ggc gaa gct gtg aaa acc cac acc
 N I S E S H P N A T F S A V G E
 45 aac atc tcc gag agc cac ccc aat gcc act ttc agc gcc gtg ggt gag
 A S I C E D D W N S G E R F T C
 gcc agc atc tgc gag gat gac tgg aac tcc ggg gag agg ttc acg tgc
 50 T V T H T D L P S P L K Q T I S
 acc gtg acc cac aca gac ctg ccc tcg cca ctg aag cag acc atc tcc
 R P K G A A D Y K D D D D K *
 55 cgg ccc aag ggc gcc gcg gat tat aaa gat gat gat gat aaa taa GAA
 Sac 2 |-----FLAG-----| Eco
 TTC AGC CCG CCT AAT GAG CGG GCT TTT TTT TAA TTC ACT GGC CGT CGT

09581924-061900

Size exclusion FPLC (Superdex200) of
refolded Streptavidin- $C_H3\mu$

